# OBSERVATIONS ON A SPONTANEOUS TYPHOID-LIKE EPIDEMIC AMONG WHITE RATS.*

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## I. INTRODUCTION.

During the past winter, many of the albino rats used in this laboratory died of an acute infectious disease. Among the rats which became infected were five litters, the majority of which had been thymectomized when from 10 days to 2 weeks old, and which had been kept under close observation for several months. As these rats showed symptoms of illness, they were killed together with control, unoperated animals of the same litters, and a routine histological examination made of the principal organs. The lesions found proved to be so striking and to resemble in so many respects the lesions of human typhoid fever, that it seemed desirable to isolate the infecting organism and to study the disease experimentally.[1]

Interest in epidemics among rats and mice dates back to the first publication of Löffler[2] in 1892. Löffler isolated from spontaneously infected laboratory mice a motile, gram-negative bacillus of the typhoid-colon group to which he gave the name *B. typhi murium*. This bacillus was pathogenic for field mice, but not regularly so for house mice; grey rats could not be infected. The following year Löffler used cultures of this organism to combat a plague of field mice which were ravaging the crops in

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[1] A preliminary report of our work was presented at the meeting of the New York Pathological Society in May.

[2] *Centralbl. f. Bakteriol.*, 1892, 11, p. 129; 1893, 13, p. 647.

Thessaly, and his successful experiences inaugurated the use of bacteria of this group for the wholesale destruction of rodents.

Danysz[1] in 1900 isolated from an epidemic of field mice in Charny-en-Seine a similar gram-negative bacillus which at first exhibited marked virulence for rats. The Danysz virus has been repeatedly studied, both as to its bacteriological relationships and as to its practical applicability to the destruction of rats.

Issatschenko,[2] Tartakowsky,[3] Schilling,[4] Trautmann,[5] and Schern[6] have described spontaneous epidemics among laboratory rats, in all of which bacilli of the typhoid-colon group have been isolated. There is further an abundant literature dealing with the interrelationships of the different strains of rat and mouse viruses, and their identity with one or another type of paratyphoid, enteritis, or hog cholera bacillus. We may refer here to the papers of Bahr,[7] Raebiger and Grosse,[8] Bainbridge,[9] Rosenow,[10] Kutscher and Meinicke,[11] Xylander,[12] Mereschowsky,[13] Bongert,[14] and Steffenhagen,[15] and shall have occasion to cite some of these workers in discussing the identity of the bacillus isolated by us.

Interest in the study of these organisms has thus centered largely upon their practical use in the extermination of rats and mice, and to this end cultures prepared by various hygienic institutes and commercial firms have been used on a large scale. Furthermore, the close relationship of the "Rattenschadlinge" to paratyphus and meat-poisoning bacilli, has suggested that their indiscriminate use might give rise to human infections, and this possibility appears to have been established by the experiences of Handson, Williams, and Klein,[16] Mayer,[17] Trommsdorf,[18] Shibayama[19] and Fleischanderl.[20]

In contrast to the abundant literature dealing with the bacteriologic and hygienic aspects of the disease are the relatively scant references to the pathological features. The gross changes, it is true, have been repeatedly described, and with considerable uniformity save as regards the intestinal lesions. On the other hand, so far as we are aware, a careful histological study has not been published. Mallory and Ordway in 1909[21] first called attention to the resemblance of the lesions produced by the injection of the Danysz bacillus in rats to the lesions of human typhoid, and demonstrated illustrative preparations at the meeting of the American Association of Pathologists and Bacteriologists. Beyond a brief abstract, no complete report of their work has appeared. During the progress of our study, there appeared a paper by Ordway, Kellert, and

[1] *Ann. d. l'Inst. Pasteur*, 1900, 14, p. 193.
[2] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1898, 23, p. 873; 1902, 31, p. 26.
[3] Ref. in *Baumgarten's Jahresber.*, 1902, 18, p. 597.
[4] *Arb. a. d. k. Gsndhtsamte*, 1902, 18, p. 108.
[5] *Ztschr. f. Hyg.*, 1906, 54, p. 104.
[6] *Arb. a. d. k. Gsndhtsamte*, 1909, 30, p. 575.
[7] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1905, 39, p. 263.
[8] *Ibid.*, 1910, 54, p. 231.
[9] *Jour. Path. and Bacteriol.*, 1909, 13, p. 443.
[10] *Bull. No. 5, Hyg. Lab., U.S. Marine Hosp. Serv.*, 1901.
[11] *Ztschr. f. Hyg. u. Infectionskrankh.*, 1905, 52, p. 301.
[12] *Ztschr. f. Fleisch- u. Milchhyg.*, 1908, 18, p. 246.
[13] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1895, 17, p. 742.
[14] Kolle u. Wassermann, *Handbuch d. Path. Mikroorg.*, Ergzheft, 1903, 3, p. 742.
[15] *Arb. a. d. k. Gsndhtsamte*, 1911, 36, p. 198.
[16] *Brit. Med. Jour.*, 1908, 2, p. 1547.
[17] *München. med. Wchnschr.*, 1905, 47, p. 2261.
[18] *Arch. f. Hyg.*, 1906, 55, p. 279.
[19] *München. med. Wchnschr.*, 1907, 64, p. 979.
[20] *Ibid.*, 1909, 56, p. 392.
[21] *Jour. Am. Med. Assn.*, 1909, 52, p. 1455.

Huested[1] on "A Typhoid-like Disease in Rabbits, Produced by the Subcutaneous Inoculation of a Strain of *B. suipestlicus*." The lesions noted by them in this experimental disease are in many respects identical with those obtained by infecting rabbits with the organism isolated by us.

## II. SYMPTOMS OF THE DISEASE.

We have, unfortunately, no data as to the percentage of infections or mortality. Of 11 young rats amply exposed to direct contact infection, 8, or 72 per cent, showed at autopsy characteristic lesions of the disease; the remaining 4 rats evidently escaped infection, or were so mildly infected that no traces of the disease could be found. The deaths in the stock cages during the winter months were very numerous, but during the late spring, no rats became spontaneously infected, the epidemic evidently having spent itself. The duration of the disease, as it occurred spontaneously, could not be accurately determined, but appeared to vary within wide limits. In several litters which had been under observation for some time and which were being weighed daily, the duration of the disease, as measured by the initial loss of weight, was 3, 4, 7, and 12 days respectively. These data are only approximate, as the animals were killed when moribund. Moreover, in two rats, characteristic lesions were found altho there had been no previous loss of weight.

In animals experimentally infected and allowed to die, the duration of the illness varied with the mode of infection. Two rats after intraperitoneal injection of a large dose died after two and three days respectively; after subcutaneous inoculation death occurred in six and seven days; after infection by feeding, in nine days.

Emaciation and loss of weight were very marked in most of the rats. There was regularly observed a marked anemia, as judged by the blanching of the ears and the pallor of the eye-grounds. Smears from the blood showed great numbers of normoblasts, and marked polychromatophilia and granular degeneration of the erythrocytes. The normal pink color of the eyes was often changed to a brownish or chocolate tinge by which the disease could be readily diagnosticated. This has been shown by Boycott[2] to be due to methemoglobinemia. A bloody crust about the nose and eyes was always present in the terminal stages. Diarrhea occurred in

[1] *Jour. Mel. Research.* 1913, 28, p. 41.

[2] *Jour. Hyg.*, 1911, 11, p. 443.

some, but by no means in the majority of the cases. Many of the rats showed no intestinal disturbances, and voided normal dry globular scybalae until their death.

### III. BACTERIOLOGICAL FINDINGS.

A bacteriological study was made of three rats dying of spontaneous infection during this epidemic. The cultures obtained were compared with one another morphologically and biologically to establish their identity, and were then inoculated into four rats and fed to two others to fulfill Koch's postulates for determining the etiological relationship of a microorganism to a disease. Other animals were also experimentally inoculated. The strains isolated were also compared with the following cultures:

1. *B. enteritidis* Gaertner, obtained from the collection of the American Museum of Natural History, New York City.
2. *B. typhi murium* A, also obtained from the American Museum of Natural History, is a subculture from a strain isolated by S. Miggi from the "Liverpool virus," a commercial rat poison sold in England and in this country.
3. *B. typhi murium* B, a strain from our laboratory collection.
4. Bacillus from the Rockefeller Institute, isolated during a rat epidemic by Dr. F. Haines.
5. *B. paratyphosus* B. (Schottmüller), from the laboratory collection.
6. *B. coli communis*, from the laboratory collection.
7. *B. typhosus* (James), from the laboratory collection.
8. *B. typhosus*, isolated by one of us from a patient with typhoid fever.

#### METHODS EMPLOYED IN THE COMPARATIVE TESTS.

Indol and nitrate cultures were examined four, six, and fifteen days after inoculation and were then kept for a few weeks. The observations on the sugar fermentation tubes were made after 48 hours. All fermentation comparisons were made with tested sugars in sugar-free broth. Four degrees of gas production were noted, namely + = trace of gas; ++ = one-fourth of closed arm full of gas; +++ = half-closed arm full of gas; ++++ = three-fourths of closed arm full of gas. Negative control tubes were used in all tests.

Agglutination tests were invariably made by the macroscopic method. Emulsions of the bacteria of approximately the same degree of opalescence were made in normal saline solution from agar cultures grown at 37° for 18 hours. The agglutination readings were taken after incubating the tubes for two hours, and again after four hours; they were then kept at room temperature, and a third reading was taken the next morning. The four-hour reading is used in the following table. In all cases the highest dilution of the serum at which agglutination was visible to the naked eye was taken as the agglutination limit of the serum. Control tubes containing only bacterial emulsion and normal saline were used in every experiment. The serum used was

monovalent and was obtained from a rabbit which had received five injections of dead, and five of live, bacteria. The injections were made daily, with four days' interval between administering the dead and the live bacteria. The bacteria used for immunization were of the strain isolated from Rat A2. Three degrees of agglutination were noted, namely, + = complete reaction; ± = partial reaction; − = negative reaction. All tests were made in duplicate.

### THE INVESTIGATION OF THE RAT EPIDEMIC.

Rat A1 had profuse diarrhea, and there were crusts of blood about eyes and nose. At autopsy, there were obtained from the heart blood, liver, and spleen pure cultures of a gram-negative bacillus. This was slender and actively motile, resembling morphologically the typhoid bacillus.

Rat A2 was examined about six hours after death. From the heart blood, liver, and spleen in all plates, pure cultures were obtained of an organism identical morphologically and culturally with the gram-negative bacillus found in Rat A1.

Similarly Rat A3 was examined and pure cultures of this organism were again obtained from the heart blood, liver, and spleen. Other rats were examined during the epidemic and gram-negative bacilli were found in all, but a complete cultural identification was not made.

The bacilli were readily isolated in pure culture from all the rats examined.

### MORPHOLOGICAL AND CULTURAL CHARACTERS.

The bacillus is slender, actively motile, gram-negative, and non-spore-bearing. It stains with the ordinary laboratory dyes. In the tissues of the rats the bacillus appeared much larger than in smears from the cultures and showed bipolar staining. On agar plates, the organism grew rapidly, forming moist grey-white colonies which were large and round with smooth borders. On agar slants the growth had the same grey-white moist appearance. The water of condensation was cloudy with a heavy sediment for the first few days; after standing for a week, this cleared up for the most part.

Gelatine stab-cultures showed a fungiform growth and were not liquefied in 30 days.

Broth was evenly clouded in 24 hours. Afterward a heavy

sediment and a thin pellicle formed. At the end of four days the sediment collected in large flakes at the bottom of the tube.

Nitrates were reduced to nitrites. In Dunham's peptone broth, there was slight indol production after four to six days in nearly all tests.

On potato, a scant grey-white growth was obtained that became visible only after three to four days.

Litmus milk at the end of 24 hours was acidified, at the end of 48 hours neutral, and at the end of 3 days alkalin. It was not coagulated.

*B. enteritidis* Gaertner and *B. typhi murium* A were compared with the organisms isolated from the rats on all the culture media just described. No marked difference was found in any of their cultural characters.

The bacilli isolated from the rats were also compared on serum water carbohydrate media and on fermentation tubes, with *B. typhi murium* A, *B. typhi murium* B, and the rat bacillus from the Rockefeller Institute. Our first series of comparisons were made in April, 1913, very soon after isolating the organisms from the rats. Fermentation tubes were then inoculated with the first subcultures of A1 and A3. A2 had been transplanted about 15 times. The organisms taken for comparison were from stock cultures. The results are as follows:

TABLE 1.

SUGAR FERMENTATION REACTIONS.

| Strains | Rat B (A1) | Rat B (A2) | Rat B (A3) | *B. typhi murium* A | *B. typhi murium* B | Rat Bacillus from Rockefeller Institute |
|---|---|---|---|---|---|---|
| Dextrose | Acid | ± | Acid | + | +++ | ++ |
| Maltose | +* | + | + | + | ++ | ++ |
| Levulose | + | + | + | ++ | ++ | ++ |
| Lactose | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin |
| Mannite | + | + | ± | + | ++ | +++ |
| Saccharose | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin |
| Dextrin | " | " | " | " | " | " |
| Galactose | + | + | + | + | ++ | ++ |

* Sign + indicates gas production in fermentation tubes; the number of + signs indicates the degree of gas production.

These results were published in our preliminary paper on this subject. Since that time, about 30 daily transplantations on

glucose agar slants have been made, and we have again compared the gas production with the results tabulated:

TABLE 2.

SUGAR FERMENTATION REACTIONS.

| Strains | Rat B (A1) | Rat B (A2) | Rat B (A3) | *B. typhi murium* A | *B. enteritidis* Gaertner | *B. typhi murium* B | Rat Bacillus from Rockefeller Institute |
|---|---|---|---|---|---|---|---|
| Dextrose | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Maltose | + | + | + | + | +++ | +++ | +++ |
| Levulose | ++ | ++ | ++ | ++ | ++ | ++++ | ++++ |
| Lactose | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin |
| Mannite | +++ | ++++ | +++ | ++++ | ++++ | ++++ | ++++ |
| Saccharose | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin | Alkalin |
| Dextrin | " | " | " | " | " | " | " |
| Galactose | ++ | +++ | ++ | ++ | ++ | ++++ | ++++ |

As may be seen by comparing the two tables, all cultures showed a greater degree of gas production after growing on glucose agar for about two months. All three strains of the rat bacillus produced abundant gas in the glucose fermentation tubes. The rat bacillus from the Rockefeller Institute and *B. typhi murium* B differed from the others only in the degree of their gas production. The three strains of our rat bacillus, *B. typhi murium* A, and *B. enteritidis* Gaertner agreed in every instance except in the degree of gas production in maltose.

AGGLUTINATION REACTIONS.

The agglutinability of our three strains of organisms was compared with strains of *B. typhosus* (James), *B. typhosus* (v. W), *B. paratyphosus* B (Schottmüller), *B. coli communis*, *B. enteritidis* Gaertner, *B. typhi murium* A, *B. typhi murium* B, and the rat bacillus from the Rockefeller Institute. The results are tabulated in Table 3.

These agglutination reactions indicate that the organisms isolated from the rats differ markedly from *B. coli*, *B. paratyphus* B, *B. typhi murium* B, and the Rockefeller Institute rat bacillus. It may also be seen that the two strains of *B. typhosus* agglutinated in quite high dilutions. This curious phenomenon has also been reported by Hübener,[1] Lebram,[2] and others. They have found that

[1] *Fleischvergiftungen u. Paratyphus Infektionen*, Berlin, 1910.

[2] *Ztschr. f. Hyg. u. Infectionskrankh.*, 1909, 44, p. 411.

TABLE 3.

AGGLUTINATION REACTIONS.

Agglutination with Serum of Rabbit Immunized with Rat Bacillus No. A2. Time of Recorded Readings: 4 Hours.

| Strains | Rat B (A1) | Rat B (A2) | Rat B (A3) | *B. typhi murium* A | *B. enteritidis* Gaertner | *B. typh.* (James) | *B. typh.* (V. W) | *B. typhi murium.* B | Rat B. Rockefeller Institute | *B. coli* | *B. paratyph.* B |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dilution | | | | | | | | | | | |
| 1– 20 | + | + | + | + | + | + | + | – | – | – | – |
| 1– 80 | + | + | + | + | + | + | + | – | – | – | – |
| 1– 120 | + | + | + | + | + | + | + | – | – | – | – |
| 1– 200 | + | + | + | + | + | + | + | – | – | – | – |
| 1– 250 | + | + | + | + | + | + | + | – | – | – | – |
| 1– 300 | + | + | + | + | + | + | + | – | – | – | – |
| 1– 600 | + | + | + | + | + | – | + | – | – | – | – |
| 1– 1,200 | + | + | + | + | + | – | – | – | – | – | – |
| 1– 2,500 | + | + | + | + | + | – | – | – | – | – | – |
| 1– 5,000 | + | + | + | + | + | – | – | – | – | – | – |
| 1–10,000 | + | + | + | + | + | – | – | – | – | – | – |
| 1–15,000 | + | + | ± | + | ± | – | – | – | – | – | – |
| Saline Control | – | – | – | – | – | – | – | – | – | – | – |

the typhoid bacillus at times agglutinates almost up to the titer limit of the serum of *B. enteritidis* Gaertner.

These agglutination experiments seem to confirm the results of our culture comparisons and suggest that the organism isolated from the rats, *B. enteritidis* Gaertner, and the organism of the Liverpool virus are very closely allied.

The close relationship of the organism of the Liverpool virus and *B. enteritidis* Gaertner has previously been reported by Steffenhagen[1] who made an extensive investigation to determine this fact. He reported that they agreed in practically all respects. The work of Mühlens, Dahm and Fürst,[2] von Ermengen,[3] Schern,[4] and others suggests that both in their cultural characteristics and in their agglutination reactions the organisms isolated by them from rat epidemics agreed with the Danysz, Ratin, Issatschenko, and Gaertner bacillus.

Bainbridge[5] also reports that *B. enteritidis* Gaertner and the Danysz bacillus, which can be easily distinguished from *B. paratyphus* A, *B. paratyphus* B, *B. Aertryck*, and *B. suipesticus* by their agglutination reactions, are indistinguishable from one another

[1] *Op. cit.*

[2] *Centralbl. f. Bakteriol.*, I, *Orig.*, 1909, 48, p. 1.

[3] Kolle u. Wassermann, *Handbuch d. Path. Mikroorg.*, 1903, 2, p. 637.

[4] *Op. cit.*

[5] *Op. cit.*

and apparently, also, are merely strains of the same organism. He also reports that *B. typhi murium* has no existence as a definite organism, since different strains alleged to be *B. typhi murium* and obtained from accredited sources were found to differ greatly. His reports seem to agree with our findings.

It is therefore evident that the bacillus which we have isolated from this epidemic agrees in nearly all respects with *B. enteritidis* Gaertner and with *B. typhi murium* A which we secured from the American Museum of Natural History and which is a subculture of a strain originally isolated from the Liverpool virus, a commercial rat poison sold in England and this country.

This Liverpool virus has also been investigated by Handson, Williams, and Klein.[1] An epidemic broke out in a large business house in London. Twelve persons were taken ill with the disease. Ten days later when they were all convalescent, an investigation revealed the fact that, altho these men all ate at the business house with many other employees, only those who ate in a certain room were taken ill. In this room a bad odor was noticed, and upon removal of the floor boards, 40 dead rats were found. It was then discovered that a rat poison, the Liverpool virus, had been spread on bread and placed around the room so that the rats could get it. Cultures were made from the dead rats and from the patients, and Klein reports that the bacilli isolated agreed in every respect with each other, and with the organism found in the Liverpool virus. Blood sera of the convalescent patients agglutinated the organisms of the Liverpool virus, the organism isolated from the dead rats, and the organism isolated from the patients.

During an investigation of mouse typhoid, Mayer,[2] who was conducting the work, was taken violently ill. Upon examination of his stools mouse typhoid bacilli were isolated. His blood serum in high dilutions agglutinated the mouse typhoid bacilli. He therefore concludes that mouse typhoid bacilli can cause acute and violent illness in man.

Four separate outbreaks of gastro-enteritis, with one death, have been observed by Shibayama[3] in Japan, who shows reasonable grounds for attributing them to the careless use of a rat virus containing Löffler's *B. typhi murium.*

[1] *Op. cit.* [2] *Op. cit.* [3] *Op. cit.*

ANIMAL INOCULATIONS.

In order to establish the etiological relationship of the organism to the disease, we inoculated six rats brought into the laboratory especially for these tests from a lot that had no record of disease among them and which were kept under observation for several days.

Two rats were inoculated intraperitoneally with 0.5 c.c. of a saline suspension of the bacilli grown on agar for 24 hours. These rats died in two and three days. They developed no characteristic symptoms.

Two others were inoculated subcutaneously with the same amount of bacterial suspension; they had no diarrhea, but bloody crusts about eyes and nose formed in five days and the animals were dead in six and seven days.

The last two were fed with bread soaked in 24-hour broth culture; they developed all the characteristic symptoms and died in nine days.

At autopsy, the organisms were recovered in pure culture in all six experimental tests from the heart blood, liver, and spleen, and the characteristic lesions were present.

These results therefore clearly establish the etiological relationship of the bacilli to the disease.

We then inoculated a rabbit, a guinea-pig, and two mice, one intraperitoneally and one subcutaneously with a saline suspension of the bacilli. One c.c. of the bacterial suspension killed a rabbit in three days, 1 c.c. killed the guinea-pig in 18 hours, and one-third of a cubic centimeter killed the two mice in 18 hours.

In every case, the organisms were recovered in pure culture and in smears from the heart blood of the animals; large numbers of the bacilli were found, showing that the organism had developed, giving rise to a true infection. It was difficult to find the bacilli in smears from the heart blood of the rats dying of spontaneous infections, altho they grew in cultures. We also inoculated a rabbit with the same amount of bacterial suspension which had been given to the other animals, killed by heat, as a control for the virulent inoculated material of the cultures. The rabbit was not killed.

As yet we have not tried to infect other animals by feeding.

The majority of workers, however, have not been able to reproduce the disease in rabbits, cats, and dogs by feeding them with the bacillus isolated from epidemics in rats.

### CONCLUSIONS.

The organisms isolated from the rats proved to be etiologically concerned in causing the epidemic. They were found to agree with the bacillus of the Liverpool virus and *B. enteritidis* Gaertner, both in their cultural characters and in their agglutination reactions. This suggests the probability that all these bacilli are very closely related to one another, if not strains of the same organism. It would seem, further, that the use of commercial rat viruses containing these organisms may be a menace to man.

## IV. PATHOLOGICAL FINDINGS.

### 1. PREVIOUS OBSERVATIONS.

The lesions observed by Löffler[1] in the mouse epidemic described by him were enlargement of the spleen, necrotic foci in the liver, and hemorrhagic gastro-enteritis. The Peyer's plaques and the mesenteric lymph-nodes were swollen and congested. Masses of bacilli were always found in the necrotic areas in the liver, but occurred also in the liver capillaries apart from the necroses.

Schilling[2] in 1902 published an account of a spontaneous epidemic among laboratory rats. The duration of the illness was only one or two days, the rats showing weakness, roughness of the hair, closing of the eyes by crusts, and in most cases diarrhea. Retardation of growth was observed in those rats which escaped the disease. Pathologically, there was found enlargement of the spleen. The stomach was normal, but the middle portion of the small intestine exhibited various grades of inflammation and edema, with swelling of the follicles. In the mild cases, the intestinal contents consisted of thin fluid, with gas bubbles; in the severe cases, of mucus admixed with blood. The lower portion of the small intestine, the cecum, and colon were unchanged. The lungs in the acute cases, showed merely petechial hemorrhages and small areas of collapse, but in the more protracted forms of the disease, the lungs were dense, infiltrated, and contained yellowish areas of necrosis. Schilling thus distinguishes two forms of the disease, one acute, with predominantly intestinal lesions, the other more chronic, with marked alterations of the lungs.

Trommsdorf[3] in 1903 gave a fairly detailed description of the lesions produced in white rats by feeding bacilli isolated from the feces of patients suffering from an epidemic of dysentery. These bacilli were agglutinated by mouse-typhoid serum, and were considered by Trommsdorf to be identical with *B. typhi murium.*

The spleen in the infected mice was enormously enlarged, and usually firm. The liver was dark red and showed fatty areas, or was sprinkled with yellowish points. The kidneys were normal. The small intestine was edematous, injected, and filled

[1] *Op. cit.* [2] *Op. cit.* [3] *Op. cit.*

with fluid feces. The mucosa was sprinkled with hemorrhages. The mesenteric, inguinal, and axillary lymph-nodes were swollen and hemorrhagic. The lungs were normal. Bacilli were found in smears from liver and spleen and in cultures from all organs.

Bahr[1] in 1905 made a comparative study of the Danysz and Issatschenko viruses, of a commercial virus called "Ratin," and of *B. typhi murium*. Incidentally, he studied the lesions produced by feeding brown rats with cultures of "Ratin." The lesions noted are the same as those recorded by previous observers, save that Bahr mentions the occurrence of fibrinous peritonitis.

Trautmann[2] in 1906 described the lesions found in epidemic and sporadic infections among laboratory rats at the Hamburg Hospital, and emphasized the resemblance of the clinical picture to that of rat plague. The autopsy findings in both spontaneously and experimentally infected rats were emaciation, hemorrhagic bubos, swelling and punctiform necroses of the liver, marked enlargement of the spleen, hyperemia of the lungs, often with necrotic foci. In feeding experiments, there was swelling of the Peyer's plaques and of the mesenteric, retroperitoneal, and submaxillary lymph-nodes. No microscopic studies were made.

In 1906, there appeared also an exhaustive paper by Kutscher and Meinicke,[3] dealing with the comparative cultural and biological characters of numerous strains of *B. paratyphus* A and B, *B. enteritidis* Gaertner, and *B. typhi murium*. The pathogenicity of the various strains was tested on mice, rabbits, and guinea-pigs, but aside from the gross lesions noted by previous observers, no detailed pathological descriptions are given.

Schern[4] in 1909 studied an epidemic among tame laboratory rats in which the obvious signs of illness lasted only two to three days. The disease was characterized by emaciation, loss of appetite, and in some animals by profuse diarrhea. The lesions found were injection of the peritoneum, without inflammatory exudate, marked enlargement of the spleen, cloudy swelling of the liver, with punctiform red areas beneath the capsule and on section. In the spleen and liver of animals which survived the infection for a longer period, there were pinhead-sized greyish or greyish-yellow foci, suggesting tubercles. The stomach was normal. The intestine was distended, the mucosa in the greater number of cases swollen and congested. The Peyer's plaques were conspicuous. Kidneys were cloudy and swollen, the adrenals slightly reddened. In two rats, large nodules suggesting "pseudo-tubercles" were present in the lungs.

Steffenhagen[5] in 1911 made a careful study of the gross lesions in rats fed with the Liverpool virus, which he considers identical with *B. enteritidis*. Like all the observers quoted, he records marked enlargement of the spleen, which is described as greyish black or reddish grey, with a firm "amyloid-like" consistence; rarely, there was found a soft, hyperplastic splenic tumor. The intestines were distended, the venules of the serosa and mesentery injected. The small intestine contained pale-yellow or thin dark-brown fluid, in which red blood cells could be found microscopically. The mucosa was unchanged but the follicles were swollen. The large intestine was not altered. The lungs were dirty grey on section, very bloody, and succulent. There were pinhead-sized hemorrhages beneath the pleura, and often greyish nodules suggesting tubercles. These consisted of necrotic areas with round-celled infiltration.

[1] *Op. cit.*
[2] *Op. cit.*
[3] *Op. cit.*
[4] *Op. cit.*
[5] *Op. cit.*

Here and there were found pepper-grain sized abscesses with minute metastatic abscesses in the surrounding area. Bacilli were not found in smears from these, so that the origin of the pulmonary lesions was questionable. The liver was enlarged, brownish red, bloody on section, showing irregular, yellowish patches; in advanced cases, the entire liver was of a bright yellow color save for small reddish patches. The cervical lymph-nodes were found enlarged in one case. The adrenals were either diffusely hyperemic or showed a hyperemic zone about the medullary portion.

Boycott[1] in 1911 made the interesting observation that infection of white rats with the Gaertner bacillus was frequently accompanied by methemoglobinemia. The other lesions noted by him, were necroses of the liver and spleen, and, in five rats, acute suppurative myocarditis of the left ventricle.

The foregoing citations include, we believe, the more important contributions to the pathology of the disease caused in rats and mice by bacilli of the paratyphoid and enteritidis group. To this list should be added the study of the liver lesions in rabbits and white mice following infections with *B. suipestifer*, by Boxmeyer,[2] and the recent work of Ordway, Kellert, and Huested, also with a strain of the hog-cholera group. These we shall consider in discussing the genesis of the liver lesions.

### 2. GROSS LESIONS.

The gross lesions noted in the present epidemic agree quite closely with those recorded by the writers cited above. No differences were observed between the spontaneously and the artificially infected rats, nor between those infected by subcutaneous injections and those in which the disease was reproduced by feeding. The intestinal lesions, in particular, were not more striking in animals infected by the intestinal route.

Emaciation was always more or less marked. The peritoneal cavity was always free from exudate, even in rats injected intraperitoneally. Marked hyperemia, as described by Bahr and Schern, was not seen. The liver was pale and friable, and sometimes mottled. In some cases, minute, translucent, greyish foci could be detected with the magnifying glass, but were not visible to the unaided eye. The spleen was greatly enlarged in all cases but one, in which it measured only 27 mm. as against 33 mm. and 32 mm. in the healthy controls of the same litter. This exceptional finding will be discussed later. A typical example is Litter D, in which the infected spleens measured 37, 40, and 39 mm.; that of a healthy control rat of the same litter, killed at the same time, 23 mm. The spleens were firm and tense, very dark bluish purple, but irregularly mottled on section by hemorrhagic and greyish areas.

[1] *Op. cit.*

[2] *Jour. Med. Research*, 1903, N.S., 4, p. 146.

The stomach showed nothing abnormal. The intestines were usually collapsed. The intestinal contents in those animals which had previously had diarrhea were fluid, sometimes admixed with mucus, and occasionally flecked with bright blood. Other animals showed normal intestinal contents, with formed scybalae in the large gut. A few scattered petechial hemorrhages were occasionally seen, but no lesions which could be justly described as a hemorrhagic enteritis. The appearance of the Peyer's plaques varied. In the majority of the cases, they were pale and not abnormally prominent. In one case, only, was there found a small ulcer, and this proved on microscopical examination to be a localized destruction of mucous membrane by a rather extensive submucous hemorrhage.

The mesenteric lymph-nodes were not noticeably enlarged, and were not hyperemic. The lungs, with the exception of occasional small purpuric spots beneath the pleura, showed no gross change. One rat, which had been infected by feeding, showed marked alterations in one lung, the relation of which to the inciting organism was not proven. The lung in this case was shrunken, firm, and riddled with caseous abscesses of large size. Microscopically, there was an interstitial pneumonia, and bronchiectatic cavities filled with necrotic exudate. Whether this isolated finding is related to the disease, we are unable to say, as no bacteriological examination of the lung was made, altho the organism was readily recoverable from the blood and spleen in this case. The lesion is apparently the same as that described by Schilling,[1] and according to Currie,[2] is a not infrequent finding in rats apart from the disease which we have studied. The fact that a similar bronchiectatic cavity, lined with squamous epithelium, was found in a rat killed three days after infection would make it improbable that the lesion is associated with the disease.

The heart muscle was sometimes pale, and in one spontaneously infected rat showed macroscopically visible opaque, greyish areas in the left ventricle. The blood was often strikingly thin, and sometimes of a distinct brownish tinge.

Swelling of the cervical and mediastinal lymph-nodes was quite

[1] *Op. cit.*

[2] *Bull. No. 30, U.S. Pub. Health and Mar. Hosp. Serv.*, 1910, p. 55.

regularly found. No gross changes were observed in the kidneys, pancreas, testes, adrenals, or brain. The thymus was usually atrophied and the interlobular septa edematous.

### 3. HISTOLOGICAL LESIONS.

*Liver.*—In both spontaneously and experimentally diseased rats the liver was the seat of numerous focal necroses. These were present in all the cases examined, with the exception of three rats killed on the day following subcutaneous inoculation. The smallest and earliest lesions (30 hours after injection) appeared as agglomerations of distorted nuclei, lying in a fibrinous meshwork in which were entangled a few red blood cells. In the older lesions, the nuclear constituents gradually disappear; there remains a sharply outlined, roughly circular area, almost devoid of cells, staining intensely with eosin, and showing a swollen nodular framework of fibrin. In places the interlacing nodular strands of fibrin appear to fuse into hyaline masses. The fibrinous material stains orange red with Mallory's connective tissue stain, and bluish with Weigert's fibrin stain.

The necrotic areas are at once made evident, in sections stained with Sudan III, by the abundant accumulation of fat drops. These are of largest size and most numerous at the margin of the necroses. The central portion may contain a few finely divided droplets, or may be virtually free from fat. Here and there are small groups of liver cells laden with fat. These are probably at the edge of necrotic areas which did not appear in the sections. Fine fat droplets are also found free in the capillaries and within the Kupffer cells.

Apart from the necrotic areas, the liver cells show no degenerative changes. In some of the rats, notably in one killed 24 hours after subcutaneous inoculation, mitoses are very numerous, but not especially so about the necroses.

Within the liver capillaries, are found in great number abnormal cellular elements and occasional fibrin thrombi. The cellular structures are, for the most part, large mononuclear cells with basophilic cytoplasm, and relatively large, kidney-shaped nuclei. The fewest of these are normal and the greater number more or less

degenerated. They very commonly inclose pyknotic nuclear particles; sometimes the fairly preserved remnant of a lymphocyte, sometimes one or more red cells, rarely a group of bacilli. One finds also unphagocyted chromatin fragments and small cells with ring- or signet-shaped nuclei, obviously lymphocytes in various stages of

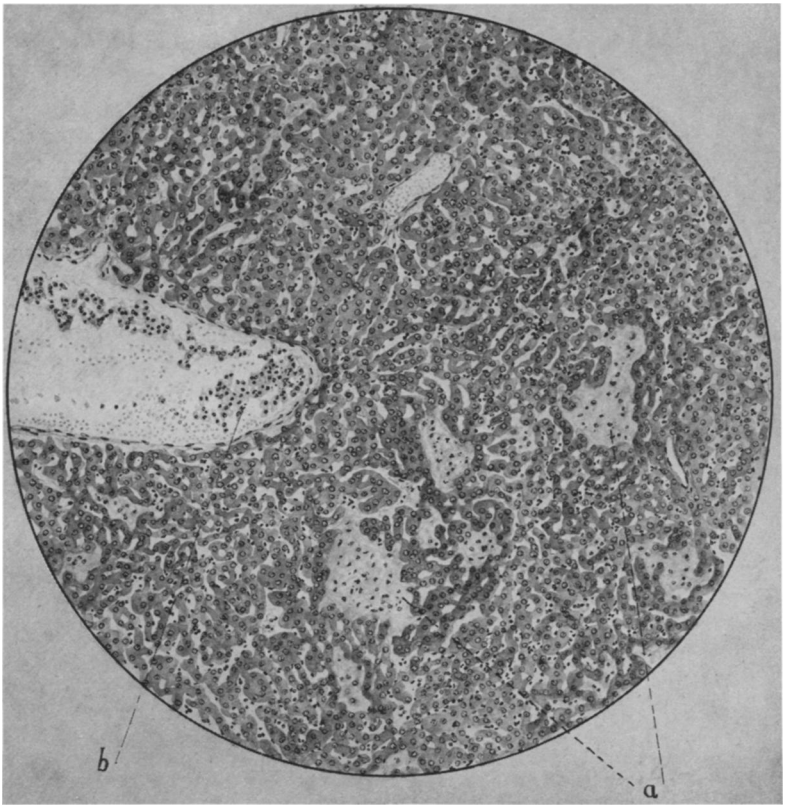


FIG. 1.—Liver of *Rat C1*, spontaneously infected, showing necrotic areas (*a*), and large masses of cells in a sub-lobular vein (*b*).

pyknosis. In some of the sections, cellular elements and detritus of this sort are extremely abundant, and appear to block the capillaries, especially when they become inclosed in a fibrinous matrix. We shall discuss the relation of these cell fragment thrombi to the origin of the necroses farther on.

The behavior of the Kupffer cells was somewhat variable. In some of the rats they were swollen and vacuolated, sometimes exfoliated, and frequently contained inclusions of nuclear material or erythrocytes. In other rats showing equally extensive areas of necrosis, the Kupffer cells showed little or no change. In one infected rat which was vitally stained with Trypanblau very few

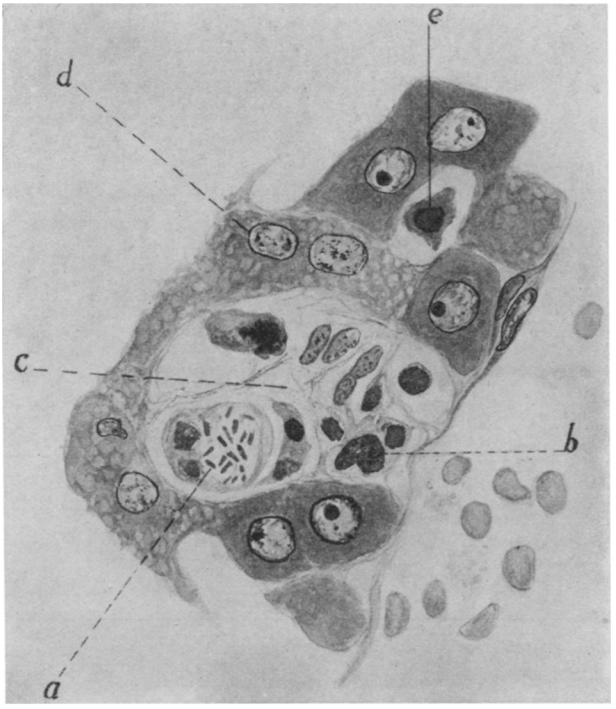


FIG. 2.—Early cell fragment thrombus in liver. At (*a*) mononuclear phagocyte enclosing bacilli; *b*) degenerating mononuclear cells; (*c*) fibrin net.

of the cells lying free in the capillary lumina contained blue granulae. There was no noticeable accumulation of the stained cells about the necroses or within them. Those endothelial cells which lay within the necrotic areas were obviously degenerated. The blue-staining material was in the form of large clumps instead of granulae of fairly uniform size. So far as one can judge from this single experiment, it would seem that the Kupffer cells are apparently not

the chief source of the foreign cellular elements and cell detritus in the capillaries.

All the different forms of cell fragments, phagocytes, and intact mononuclear cells are found in the portal vein, and at times in the efferent veins of the lobules. They occur singly or in large

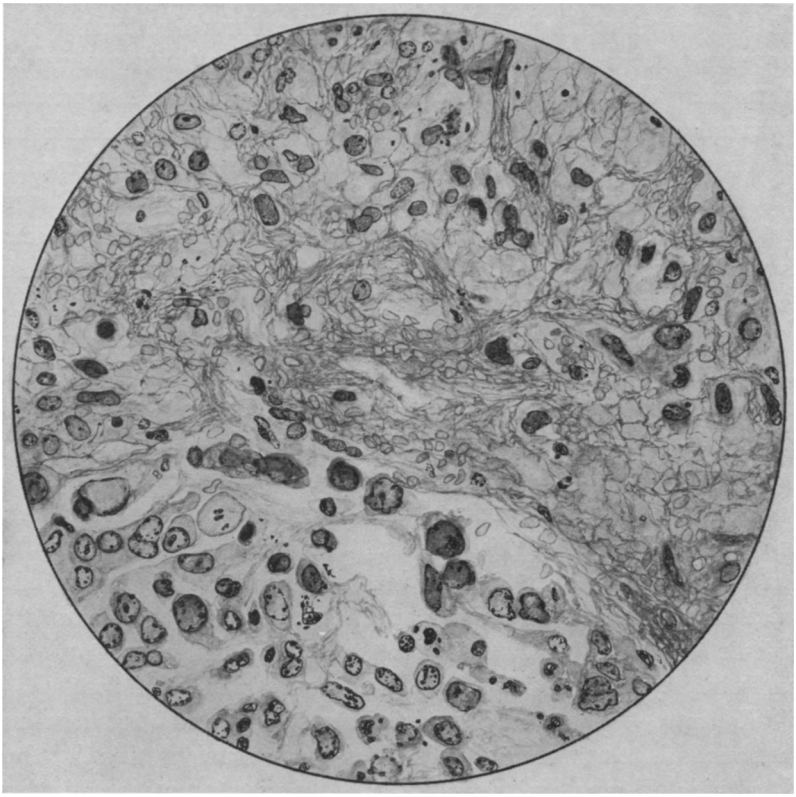

FIG. 3.—Spleen of *Rat B-2-x*, experimentally infected. Section includes edge of follicle. Note fragmentation of nuclei, especially in pulp, and abundant exudation of fibrin.

clumps, in which the cellular constituents are imbedded in a fibrinous matrix.

*Spleen.*—In this organ, the lesions are localized in the early stages, more diffuse in animals which survive the infection for a longer period. The essential, and probably the primary change appears to be an intense destruction of the lymphoid cells both with-

in and without the follicles. Throughout the pulp, one finds small mononuclear cells in all stages of disintegration, as evidenced by the abundance of fragmented chromatin. Much of this nuclear detritus is within macrophages, which in turn undergo degeneration. The necrosis of the pulp cells is accompanied by a great outpouring of fibrin, forming a nodular network about the degenerating cells. The filaments of fibrin appear to use the fibrous reticulum as a support. There is often distinct hemorrhage, but rarely excessive pigment. The follicles show invariably a more or less marked pyknosis of the small lymphoid cells. This is accompanied in the earlier stages by an excessive formation of large mononuclear cells. The number of mitoses among these may be extraordinary. Phagocytosis of chromatin fragments by the larger cells of the follicles is of course regularly seen. No fibrin could be demonstrated within the follicles.

Bacilli are found in small groups or in larger clumps both within and without the sinuses. In spontaneously infected rats, or in those infected by feeding, very few bacteria may be present. The sinuses are crowded with red cells, but they may contain impacted masses of mononuclear cells or cell-débris, about which there may be formed a definite fibrin thrombus. Masses of cells are also commonly found in the splenic veins. Among these are cells which may undergo mitotic division in the blood stream. Such cells were also found several times in smears made directly from the splenic blood, in which one finds also numbers of nuclear fragments and phagocytes enclosing red cells and chromatin particles.

*Kidneys.*—In animals experimentally infected with subcutaneous or intraperitoneal injections, it is usual to find hyaline thrombi in the glomerular capillaries. Every tuft may show a more or less complete blocking. The appearance is especially striking in sections stained with Mallory's anilin blue, the hyaline material taking a reddish-orange color in contrast to the yellow of the red blood cells. Similar thrombi may be found in the capillaries of the medulla and pyramids.

Bacterial emboli in the glomerular loops and in the capillaries between the tubules were seen in two rats inoculated intraperitoneally, but were not regularly present.

*Myocardium.*—Lesions in the myocardium were found both in the spontaneously and experimentally infected rats, tho not in all cases. The changes were in the nature of an acute interstitial inflammation, and occurred by preference in the superficial strata

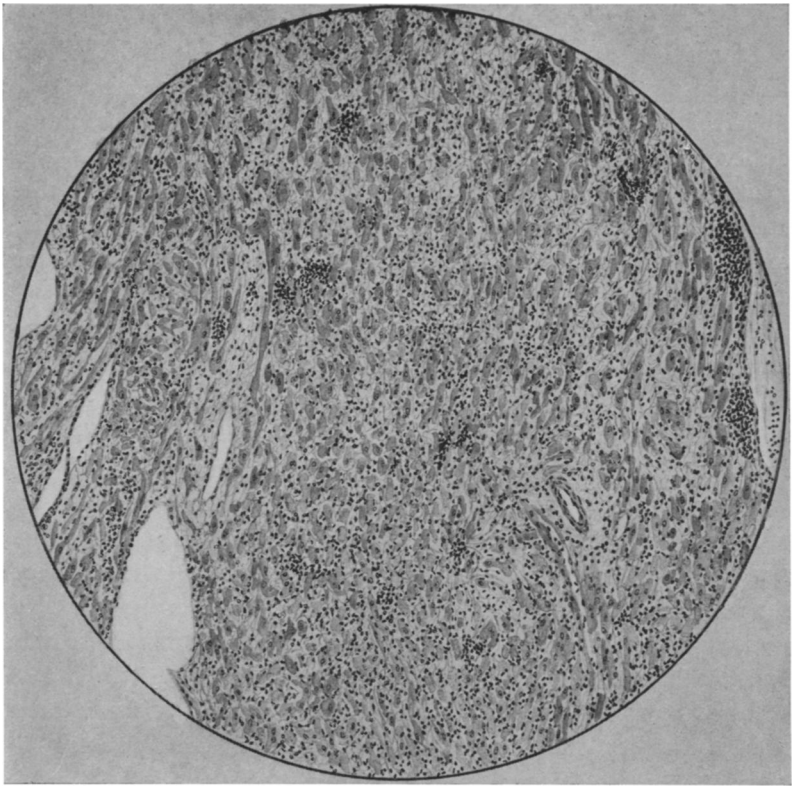

FIG. 4.—Myocardium of left ventricle, showing acute interstitial inflammation in spontaneously infected rat.

of fibers, more especially in the left ventricle. Rat A (spontaneously infected) showed extremely marked lesions (Fig. 4). The fibers were forced apart by a cellular exudate and fibrin. Most of the inflammatory cells are fragmented beyond recognition, but at the margin of the lesion, it can be seen that there is swelling and degeneration of the fixed connective tissue cells, as well as a moderate infiltration of lymphoid and polymorphonuclear elements.

The muscle fibers show various degrees of degeneration up to complete necrosis. In some areas, the fibers have disappeared completely, leaving only an irregular eosin-staining material, in which are found swollen, distorted nuclei and chromatin fragments. Bacilli occurring singly and in clumps are found without difficulty between the muscle fibers.

*Lungs.*—The septa appear relatively stout and more cellular than those of normal lungs. This is due to the stuffing of the capillaries with large mononuclear cells similar to those found in the liver. Many of the larger pulmonary arteries contain groups or larger masses of these cells. Pneumonic lesions, aside from these interstitial changes, were not found, and the bronchi were free from exudate. The one exception (Rat D2*x*) has already been described.

*Stomach and intestines.*—The only lesions found in the stomach were occasional small hemorrhages into the mucosa. The intestinal lesions, on the whole, were not striking, and in some of the rats no definite alterations were found. In others there was moderate fragmentation of the lymphoid cells, with phagocytosis by the swollen reticular elements. The overlying mucosa was usually intact, but not infrequently the villi were edematous, and there were more or less extensive hemorrhages; this had led in one case to a separation and desquamation of the epithelial covering; aside from this purely mechanical loss of substance, no ulcers were found in any of the rats examined.

*Lymph-nodes.*—Serial sections of the neck organs were made from a number of the spontaneously infected rats for another purpose. In all the cases it was found that the cervical lymph-nodes were greatly enlarged and the seat of extensive necroses similar to those described in the spleen. Altho the thymus exhibited advanced involutional changes such as are found in all infectious diseases accompanied by wasting, in no case did it contain areas of necrosis, even when all the surrounding lymphoid tissue, including that which abundantly envelops the primary bronchi, was extensively affected. The mesenteric lymph-nodes showed slight involvement in some rats; in others, no changes were present.

The marrow of the long bones contained more or less extensive

areas of necrosis (Fig. 5) distributed throughout the shaft. Necroses were also found in the bones of the jaw and clavicle. There seemed in some cases to be an increased number of megakaryocytes present, and in smears from the marrow

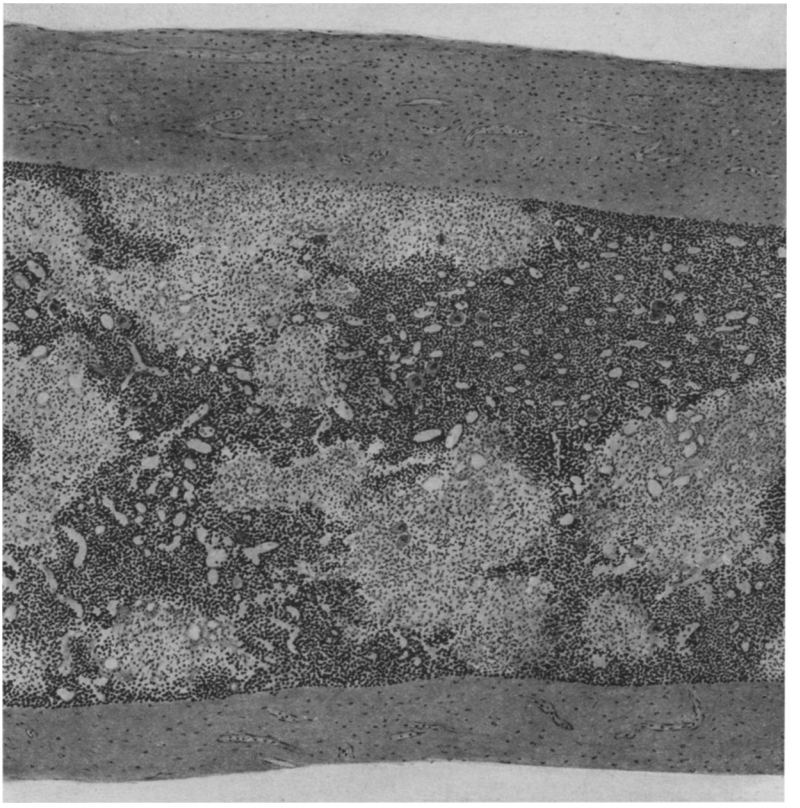

FIG. 5.—Section through shaft of femur showing extensive necroses in marrow of *Rat D-4-x*, spontaneously infected.

stained with Giemsa one of these cells containing plural mitoses was seen.

No lesions were found in the thyroid, parathyroid, pancreas, or testes. The adrenals showed no loss of chromaffinity and were normal in other respects. The central nervous system, which showed no gross changes, was not studied microscopically.

PROTOCOLS.

*Rat C-1.*—This was one of a litter which had been operated upon when 14 days old. The animal gained normally until it became ill, when the weight dropped from 98 gm. to 77.5 gm. The rat became emaciated and refused food, and the eyes were closed with secretion. It was killed by chloroform.

Autopsy: No intestinal lesions were noted. The spleen was enlarged and showed prominent follicles. The liver was large and pale. No other gross changes were observed.

HISTOLOGICAL EXAMINATION.

Liver: Very numerous areas of necrosis, for the most part discrete, but in places confluent. The liver cells at the margin of these finely vacuolated. The necrotic areas are almost devoid of cells, being composed of a coarse fibrinous reticulum, in which are a few lobulated nuclei. In some places, small accumulations of leukocytes, chiefly polymorphonuclear. Dense aggregations of cells in the portal spaces, most of them lymphoid and plasma cells. These often extend in streaks along the interlobular bile-ducts and vessels. The liver capillaries are narrowed apparently by the swelling of the liver cells. They contain degenerated cells which in a few places form definite plugs.

Spleen: With the low power, one sees numerous scattered, rather ill-defined areas, less cellular than the surrounding pulp, composed of a hyaline or granular eosin-staining material deposited in the reticulum about the splenic sinuses. The cells which are included in these regions are pyknotic and fragmented. In places, distinct hyaline thrombi occupy the sinuses in the center of these rarefied areas. About such thrombosed sinuses there are often irregular hemorrhages. The malphigian follicles show slight changes, but phagocytosis of fragmented nuclei is occasionally seen. Mitoses are not numerous. A few megakaryocytes are scattered through the pulp.

Adrenals: Normal. The chromaffin staining is marked.

Kidney: Normal.

Lungs: The alveolar walls thickened showing nodular areas of necrosis. Many of the smaller arteries packed with masses of mononuclear cells.

*Rat C-2.*—Thymectomy at 14 days. Recovered from operation, and gained steadily in weight until 24 days before death. The weight then remained stationary for 10 days, but fell from 59 gm. to 43.5 gm. during the last two weeks of life. The animal was found dead and partially eaten by the other rats of the litter.

Autopsy: No gross lesions were noted, aside from the extreme emaciation. The spleen measured only 25 mm. in length.

HISTOLOGICAL EXAMINATION.

Liver: Necroses, very abundant and some of them of large size, are composed of a swollen hyaline fibrinous reticulum inclosing a sparse number of fragmented or distorted nuclei and a few red blood cells. The liver capillaries, which are narrow, contain many small chromatin fragments, but no very definite thrombi, nor are larger masses of cells found in the portal veins. The intact liver cells often show large, hyperchromatic nuclei.

Spleen: Very markedly altered, the follicles small and very irregular. The small cells of the follicles are in all stages of pyknotic degeneration. Many large phagocytes filled with nuclear fragments. The pulp shows a striking paucity of cells as compared

with the normal. Between the compressed, and in places, obliterated sinuses, there is a large amount of a granular fibrinous material, imbedded in which are nuclear fragments and red blood cells. The endothelium of the sinuses is well preserved, but the cellular constituents of the pulp are largely disintegrated. Some of the larger sinuses contain hyaline thrombi, others are filled with loose masses of degenerating cells.

Adrenals: Almost complete loss of chromaffin staining, possibly the effect of postmortem change. The cortex is not altered.

Myocardium: In the wall of the left ventricle several small localized areas of hyaline or waxy degeneration of the muscle fibers, and about these, slight cellular infiltration.

Lungs: No noteworthy changes.

Cervical lymph-nodes: Numerous areas of necrosis, located chiefly in the lymph-cords.

In this animal, the disease was apparently of long standing, and had led to extreme destruction of the cellular elements of the spleen, and to very extensive necroses in the liver. There was, however, no attempt at healing or encapsulation, and the lesions may be considered as progressive. An exceptional feature in this rat is the small size of the spleen, due possibly to the advanced destruction of the cellular constituents, or to a secondary contraction of the necrotic areas, such as occurs in an anemic infarct.

The third rat of this litter illustrates the more acute lesions.

*Rat C-7.*—A control rat, unoperated, which gained weight progressively until killed, showing no abnormal symptoms.

Autopsy: Weight 70.5 gm. The organs appeared normal with the exception of the spleen, which measured 35 mm. in length. The follicles were very conspicuous.

HISTOLOGICAL EXAMINATION.

Liver: A few typical early necroses, some of which are the seat of marked leukocytic infiltration. Definite hyaline plugs are found in some of the necrotic foci. The small sub-lobular veins are surrounded by a mantle of lymphoid and plasma cells. Swelling and some vacuolization of the liver cells. The capillaries are narrow and contain moderate numbers of small cells, but no larger masses of cell fragments, or phagocytes.

Spleen: The pulp intensely congested, and in places, hemorrhagic. A few small areas of necrosis, with fragmentation of the nuclei. The follicles are large, the follicle cells well-preserved. Numerous megakaryocytes are present.

Adrenals: Intense chromaffin staining.

Thymus: Normal.

*Rat E-6.*—Unoperated control, aged 2 months and 6 days. Duration of disease as estimated from beginning loss of weight was 9 days, during which period the weight fell from 65.5 gm. to 59 gm. The rat was chloroformed, having shown the usual symptoms.

Autopsy: Emaciated. No peritoneal changes. Spleen much enlarged (36 mm.), with conspicuous follicles. Liver shows nothing abnormal. Adrenals, kidneys, lungs, intestines free from gross changes.

HISTOLOGICAL EXAMINATION.

Liver: Sharply circumscribed, very numerous necroses, some of large size, composed of a coarse fibrinous meshwork with occasional red blood cells, and degenerating

cells and nuclear fragments. Such cells are also found free in the capillaries, singly or massed into clumps and enveloped in a delicate fibrinous mesh. The Kupffer cells in places are swollen and contain ingested red blood cells and chromatin particles. Aggregations of large cells in the portal veins and often in the lymph-spaces of Glissons capsule.

Spleen: The section shows numerous scattered areas of necrosis, consisting of a fibrin meshwork inclosing distorted, pale, epithelioid nuclei, erythrocytes, and small particles of fragmented nuclear material. Elsewhere the sinuses are congested and frequently contain masses of large mononuclear cells, few polymorphonuclear leukocytes, and other cell fragments whose origin it is impossible to determine. Definite thrombus formation is not noted. Mitoses numerous, both within the follicles and in the reticulum. A great many megakaryocytes.

Lungs: Throughout the section, irregular, somewhat nodular areas of consolidation, not of the type of a lobular pneumonia, but consisting of cellular infiltrations or proliferations of the alveolar wall, without exudation into the alveoli. In the center of such an area, there may often be found a small pulmonary artery packed with cells which are of the same kind as those seen in the liver capillaries. Large mononuclear cells predominate. They are often vacuolated, and frequently inclose erythrocytes or pyknotic nuclear fragments. The consolidated areas are thus rather definitely distributed about the smaller arterial branches, and not the bronchi which are free from exudate and otherwise quite normal. The capillaries in these areas are not infrequently found plugged by fibrin masses inclosing nuclear fragments.

Myocardium: Most extensive changes (Fig. 4). The fibers are widely separated, there having been evidently an extreme edema of the interstitial tissue. The muscle fibers are thin, and very refractile and waxy in appearance, with complete loss of the transverse striations, and only a suggestion here and there of the longitudinal fibrils. The nuclei are for the most part still fairly well preserved, a few only being pyknotic. Between the fibers are accumulations of wandering cells, the majority of the mononuclear variety. In places, and especially beneath the endocardium, they form dense clusters. There appears to be also a proliferation of the fixed connective tissue cells; altho no mitoses are found, many of the cells between the degenerated fibers are of the fibro-blastic type. These myocardial changes are not uniformly present throughout the heart, but affect particularly the wall of the left ventricle and the papillary muscles.

Organs of the neck: These were cut in series of 10 microns. The thymus was the seat of marked involutional changes. Differentiation between cortex and medulla was lost. The lobules were separated by edematous tissue in which were many mast-cells. The mediastinal and cervical lymph-nodes were enlarged and contained many areas of focal necrosis. The thymus, on the other hand, was entirely free from necroses. The thyroid and parathyroids were normal. The salivary gland was normal.

Bone-marrow (femur): Scattered areas of focal necrosis, distributed throughout the shaft and present also in the lower epiphysis. Megakaryocytes appear to be present in increased numbers.

In all, 10 spontaneously infected rats were examined and a fairly complete microscopic study made. From the last rat examined, and from several other rats infected at about the same time, the bacillus which has been described above was isolated, and a further

study made of the lesions produced by experimental infection with pure cultures of the organism. As has been stated, it has been found to be easy to produce the disease, either by feeding or by subcutaneous or intraperitoneal inoculation. The resulting lesions were in all respects similar to those described in the spontaneously infected rats; the differences found in rats infected by different routes appeared to depend upon the duration of the illness rather than the mode of infection.

The following protocols of experimentally infected rats illustrate the similarity between the naturally and artificially produced disease:

*Rat B-1-x.*—Inoculated intraperitoneally with 0.5 c.c. of a saline suspension of a 24-hour agar culture. Found dead 48 hours later. No putrefactive changes.

Autopsy: Negative save for slight swelling of spleen. Cultures from peritoneal cavity, heart blood, spleen, and liver, positive.

HISTOLOGICAL EXAMINATION.

Liver: Many small areas of necrosis, scattered through the different portions of the lobules. The smallest, and presumably the earliest of these, appear as irregular plugs of coarse fibrin distending the capillary lumen and inclosing numerous unaltered red blood cells and a few nuclear fragments. In the capillaries are many mononuclear cells, with inclusions of masses of chromatin and red cells (Fig. 2). The Kupffer cells, swollen and vacuolated, also contain ingested nuclear particles and erythrocytes. The origin of most of these phagocytic cells, which are in all stages of disintegration, is impossible to determine. It is obvious that about these masses of cell detritus fibrin thrombi are formed in the sinusoids, and that these cell-fragment thrombi antedate the destruction of the liver cells. Thus one finds occlusion of the capillaries without noticeable alteration of the adjacent liver cells. With the stoppage of a larger capillary area, the liver cells about the thrombosed area become vacuolated, their cell outline indistinct, their nuclei shrunken and irregular in outline; the nucleolus disappears, and the chromatin lies against the nuclear membrane or is extruded into the cytoplasm. With the complete breaking-down of the liver cells, they become incorporated into the thrombotic area.

Many large mononuclear cells, most of them showing karyolytic changes, are present both in the portal and in the efferent veins. Many of the individual cells are phagocytic.

Bacilli are found in clumps in the capillaries, but in no particular relation to the necroses.

Spleen: The follicles irregular, but large and relatively well preserved. The most striking alteration is in the pulp, which even with the low power shows an extraordinary fragmentation of the nuclei (Fig. 3). The meshes of the reticulum are thickly crowded with nuclear particles of all sizes and shapes between which there is hemorrhage and fibrin formation. The picture is complicated, but it is evident that there has been an extreme destruction of the lymphoid cells, both within the follicles and in the reticulum. Coincidently there is taking place a proliferation of large mononuclear cells, with lobate or oval nuclei and basophilic cytoplasm. The follicles con-

tain many healthy-appearing cells of this type, and many of them are in mitosis. They become phagocytic for the pyknotic, small lymphoid cells and after becoming loaded with ingested nuclear material, in turn degenerate. The larger sinuses and veins contain many of these cells and some of them appear to undergo mitosis in the circulating blood. Some of the sinuses are plugged with cell-fragment thrombi, but the majority are filled with well-preserved red blood cells. The endothelium of the sinuses preserves its normal character, shows no mitoses, and contains no phagocytic inclusions. No pictures are found suggesting a differentiation of the sinus endothelium into the large basophilic mononuclear type of cell.

Large colonies of bacilli are scattered irregularly through the splenic pulp. No megakaryocytes are seen in the sections.

Kidneys: Hyaline thrombi present in the glomerular capillaries, forming more or less complete casts of the tufts. They stain intensely with Weigert's fibrin stain. The blocking of the blood-channel is not always complete, the hyaline or fibrinous material being sometimes deposited along one side of the capillary wall. The epithelium of the tuft and of Bowman's capsule unaltered. Occasional hyaline thrombi also found in the capillaries between the collecting tubules. The thrombus may extend for a distance either into efferent or afferent vessel or both.

Adrenals: Normal in all respects.

Intestines: A few hemorrhages into the mucosa. The sections do not pass through the Peyer's plaques. The serosa is free from inflammatory change.

Stomach and duodenum: Normal.

Pancreas: Normal.

Mesenteric lymph-nodes: There is a slight hemorrhage near the surface of the gland, but no necrosis. The changes are insignificant.

Lungs: By oversight, not examined. Section of lung from Rat B-2-*x* inoculated at same time and showing similar lesions in other viscera, may be taken for description. The capillaries are crowded with degenerating cells of the same character as those seen in the liver capillaries. Large mononuclear cells often enclosed in a delicate fibrin network, are also present in numbers in the smaller pulmonary arteries, but are rarely found in the veins. Bronchi and alveoli are normal.

Myocardium: The superficial layer of ventricular muscle is edematous. The fibers are separated by a fibrinous coagulum. There are bacterial emboli in the capillaries.

The following protocol describes the lesions in a rat infected by feeding.

*Rat D-1-x.*—Fed with cubes of bread soaked in saline suspension of 24-hour agar slant. No food given on following day. The rat showed the usual symptoms and died nine days after infection. On ice, six hours before autopsy.

Autopsy: Marked emaciation. Hyperemia of intestine without marked swelling of Peyer's plaques. Spleen much enlarged, liver moderately. Lungs and heart show no gross changes.

HISTOLOGICAL EXAMINATION.

Liver: Necrotic areas of considerable size present in great numbers, irregularly disposed in various portions of the liver lobules. In these areas, the architecture of the liver is destroyed; one sees merely a very coarse nodular network of fibrin in which lie a few distorted nuclei and nuclear fragments. Some of these appear to have been derived from the pre-existing endothelial cells; others are small and round and resemble

pyknotic lymphocytes. The liver cells immediately abutting on the necrotic areas sometimes show nuclear degeneration, but on the whole, the transition to normal tissue is very abrupt. Apart from the necrotic areas, one finds as in Rat B1*x*, distended capillaries filled with broken-down cellular detritus, enmeshed in fibrin, and about these, changes in the liver cells of varying degree up to complete necrosis. Very striking are the large masses of cellular detritus in the larger efferent veins.

In sections deeply stained with polychrome methylene blue, very few bacilli are found, and these scattered irregularly through the capillaries. They are often inclosed within phagocytes and degenerated. Larger bacterial colonies are not seen.

Spleen: The lesions are less marked than in many other rats examined. The follicles are small and indistinctly outlined. Fragmentation of the lymphoid cells and phagocytosis are not extreme. The changes are more pronounced in the reticulum. The sinuses which are distinct and well filled with red blood cells are widely separated by a fibrinous material, containing distorted nuclei, in different stages of disintegration. Some of the larger sinuses, but especially the larger splenic veins, are filled with masses of cells embedded in a pink-staining matrix.

Kidneys and adrenals: No marked change. No fibrin thrombi in the glomeruli.

Lungs: Lesions are practically identical with those found in Rat B2*x*.

Stomach: Localized areas of intense hyperemia in the superficial layers of mucosa, and red cells are found in numbers in the stomach contents.

Intestines: No changes are found in the mucosa of the small or large intestine. The Peyer's patches are not included in the sections.

Mesenteric lymph-nodes: Quite numerous areas of partial necrosis, in which the lymphoid cells are replaced by large cells with vesicular lobate nuclei, often containing pyknotic nuclear particles in their cytoplasm.

Myocardium: Normal.

In all, 22 rats were inoculated, and a histological study made of the lesions. The above protocols are selected as typical of the early and late stages of experimental infection. They do not, however, include a description of the changes in the Peyer's plaques, a brief record of which may be taken from the protocols of other infected rats.

*Rat M-5-1.*—Subcutaneous injection. Killed after 47 hours. Very early lesions in spleen and liver.

Stomach: Normal.

Small intestine: The epithelium is intact. Among the lymphoid cells of the Peyer's plaques are very numerous cells of large size, with vesicular, slightly irregular or lobulated nucleus. These cells are in active proliferation: mitotic figures extremely abundant. The small cells well preserved. Only a few pyknotic nuclei.

The only abnormality noted in these sections is the very active proliferation of large mononuclear cells, unaccompanied by marked destruction of the lymphocytes, or phagocytosis.

*Rat M-5-2.*—Subcutaneous injection. Killed 57 hours after inoculation. Typical marked lesions in liver and spleen. The changes in the small intestine are similar to the above, but mitoses among the large cells are less numerous.

*Rat M-12-4.*—Splenectomy. Subcutaneous infection. Killed 3 days after inoculation. Typical lesions in the liver.

Small intestine: The changes very slight, and of the same character as in Rats M-5-1 and M-5-2. No necroses or hemorrhages. The mesenteric lymph-nodes show most active proliferation of large mononuclear cells. The sinus endothelium is readily distinguished from these, and tho there is slight swelling and desquamation, no active proliferation is seen.

*Rat M-12-5.*—Subcutaneous inoculation. Killed after 3 days. Typical lesions in spleen and liver.

Intestines: Peyer's patches large. In a few places, pyknosis of lymphoid cells, with phagocytosis. The large cells show numerous mitoses. The overlying epithelium is unchanged. In some sections, however, there is hemorrhage into the mucosa, with edema as shown by the separation of cells of the stroma and the deposition of granular material. No inflammatory reaction. Sections of several taenia in the villi.

The above brief notes will suffice to show the insignificance of the intestinal lesions. Aside from moderate fragmentation of lymphoid cells active proliferation of mononuclear elements apparently derived from the reticulum, no changes of note were observed. The presence of parasites in the intestinal villi in Rat M-12-5 may perhaps explain the more marked hemorrhage and edema seen in this case.

### LESIONS IN MICE.

The following is a brief abstract of the changes noted.

*Mouse 1.*—Inoculated intraperitoneally with 0.3 c.c. of a saline suspension of a 24-hour agar slant. Found dead after 18 hours.

Autopsy: Spleen enlarged. Liver pale. Lungs congested. Many bacilli in heart blood and peritoneal exudate.

#### HISTOLOGICAL EXAMINATION.

Liver: Fibrinous exudate with large masses of bacilli on the surface. Capillaries contain increased numbers of lymphocytes with occasional pyknotic nuclei. No thrombus formation. A few colonies of bacilli in the capillaries.

Spleen: Marked degeneration of the lymphoid cells, both in follicles and in reticulum, with extremely active phagocytosis. There are also extensive hemorrhages into the splenic pulp. Many pigment-containing cells. Many megakaryocytes. No definite exudation of fibrin.

Kidney: Many bacterial emboli in the glomeruli and elsewhere. The glomerular tufts are free from fibrinous thrombi.

Myocardium: Bacterial emboli are present in the capillaries.

*Mouse 2.*—Inoculated with 0.3 c.c. of saline suspension subcutaneously. Found dying after 18 hours. Killed with chloroform.

Autopsy: Spleen enlarged and congested. No other gross lesions. Many bacilli in smears from heart blood.

HISTOLOGICAL EXAMINATION.

Liver: Same as Mouse 1. No necroses or capillary thrombi.

Spleen: Same as Mouse 1, save that large mononuclear cells in mitosis are very numerous, probably because of fresh preservation. Many megakaryocytes.

Kidneys, lungs, and myocardium are normal.

In both mice, death occurred apparently without the development of marked lesions comparable to those seen in infected rats. A third mouse, inoculated with killed cultures, exhibited marked lesions, which closely resembled those in infected rats.

*Mouse 3.*—Inoculated intraperitoneally with 0.3 c.c. of culture killed by heating. Found dead after 18 hours.

Autopsy: Spleen markedly enlarged. Liver soft and pale. Other viscera show no changes.

HISTOLOGICAL EXAMINATION.

Liver: The capillaries are packed with chromatin fragments and distorted nuclei of bizarre shape. In many places, these are associated with the formation of definite fibrin thrombi. Necroses of the liver cells are seen in many places, but the appearance differs somewhat from that found in rats in the preservation of the alignment of the necrotic cells. Their nuclei at first become pyknotic, later fragment and disappear; the cells become smaller, denser, more hyaline, and stain intensely with eosin. The necrotic areas are not very circumscribed.

Spleen: Many fairly limited areas of necrosis in the pulp, in which there is extreme nuclear fragmentation. Hemorrhages also.

Lungs: Marked hyperemia. Capillaries stuffed with nuclear fragments.

Mediastinal lymph-node: Fragmentation of lymphocytes and phagocytosis of cell detritus by large cells. Exudation of coagulable material into the lymph sinuses.

Myocardium: Normal.

No feeding experiments were performed with mice, and the intestines were not examined.

LESIONS IN RABBITS.

As was stated in the bacteriological part of this paper, rabbits were found to be susceptible to intraperitoneal and intravenous inoculations with the bacilli. The lesions produced resemble closely the lesions observed in rats, as will be evident from the following protocol.

*Rabbit 1.*—Inoculated intraperitoneally with 1 c.c. of saline suspension of 24-hour agar slant. Died in 18 hours.

Autopsy: Along lymphatics of anterior abdominal wall, several small cheesy abscesses. No free fluid in peritoneal cavity. The omentum is rolled upon itself and dotted with opaque, whitish exudate. The serosa is not greatly congested. There is fibrino-purulent exudate of a cheesy character on liver, spleen, and intestinal serosa. Liver very large, dark, with distinct lobules having a darker center. The consistence is friable. No macroscopic necroses. No coccidiosis. Spleen moderately enlarged,

dark, firm. Kidneys very soft, friable, and swollen, sprinkled superficially and on section with blotchy hemorrhages. Adrenals: medulla hyperemic. Pancreas normal. Lungs: small pleural and parenchymal hemorrhages. Myocardium, stomach, and intestines normal. Mesenteric lymph-nodes not enlarged or necrotic.

Positive cultures obtained from peritoneal cavity and heart blood.

HISTOLOGICAL EXAMINATION.

Liver: The liver cords are narrow, the capillaries wide, and filled with great numbers of cellular fragments. In many places they are agglomerated into impacted masses imbedded in fibrin, which is readily demonstrated with Weigert's stain. The appearance is identical with that repeatedly described as typical of the livers of infected rats. In addition, however, there are larger areas which have rather the appearance of irregular infarcts. The liver cells still maintain their columnar alignment, but the individual cells are homogeneous and stain intensely with eosin. The nuclei are lost, and the cells evidently completely necrotic. The blood channels in these areas are not occluded with thrombi, but in some places are plugged with masses of bacilli. The entire necrotic portion which involves several lobules, is walled off by a zone of leukocytic infiltration. In the absence of serial sections, it was not determined whether there was a blocking of larger vascular channels.

Spleen: The striking feature is the presence of dense thrombi composed of fibrin, red blood corpuscles, and nuclear fragments in many of the splenic sinuses. There are areas of hemorrhage in the pulp. The follicles show changes which are the counterpart of those noted in rats.

Lungs: There is an unorganized fibrin thrombus in one of the larger branches of the pulmonary artery. This does not show the structure of a typical platelet thrombus, but appears to be composed of irregular laminae of fibrin, and contains dense aggregations of chromatin dust, in its central portion. Similar thrombi are present in smaller arterial branches near the surface and are associated with hemorrhage into the alveoli. The capillaries are crowded with leukocytes, among them many polynuclears. There is no exudate into alveoli or bronchi.

Stomach: Normal.

Small intestine: Sections include a Peyer's plaque. The mucosa is unchanged. Rarefaction of the centers of the follicles and fragmentation of the nuclei of the lymphocytes leading to their complete disappearance in some regions. Their place is taken by large mononuclear cells, also in various stages of degeneration, and containing yellowish pigment.

Myocardium, adrenals, and kidneys show no changes.

## V. GENERAL CONCLUSIONS.

The disease, as exemplified by the foregoing description, presents features which are of considerable interest to the pathologist. The origin of the necroses in spleen, liver, lymph-nodes, and bone-marrow, which constitute the most striking alterations, demands explanation. Mallory and Ordway have already emphasized the resemblance of this rat disease to human typhoid in the character

of the lesions, and its suitability for the experimental study of some of the disputed points in the pathology of typhoid fever.

Two divergent opinions as to the origin of the liver necroses in human typhoid have been expressed. It has been held, on the one hand, that the lesions are the direct result of the action of bacterial toxins or of the bacteria themselves upon the parenchymal cells. Thus Reed[1] concludes, from a study of human lesions and necroses produced by the injection of typhoid bacilli into the mesenteric veins of rabbits, that the areas of necrosis "owe their origin to the action of the typhoid bacillus, altho it has not been possible to determine definitely in what way this cell death is brought about, that is to say, whether it is due to the immediate presence of the bacilli within the areas of necrosis, or is caused by the action of the so-called toxalbumins which are assumed to be present in the general circulation." He considers the latter more probable because of the experiments of Welch and Flexner[2] with diphtheria toxin and of Flexner[3] with minute injections of ricin.

The alternative view is that of Mallory,[4] who holds that the liver necroses in typhoid fever are the direct result of the embolic occlusion of liver capillaries by impacted masses of cells and cell-fragments, largely of endothelial origin ("endothelial leukocytes"), which are swept into the portal circulation from the spleen, intestine, and mesenteric lymph-nodes. The typhoid toxin acts primarily as a direct stimulant to the proliferation of the large mononuclear cells in these tissues. These new-formed cells are short-lived, and in their disintegration give rise to the cell fragment emboli in the liver and other organs.

In the lesions described, we have repeatedly noted the presence of great numbers of cell fragments in the liver capillaries, and of larger masses of cells in the portal vessels. Altho the appearances seemed to point to a capillary occlusion as the primary event, and the necrosis of the liver cells as a later effect, it seemed of interest to establish this point experimentally, if possible.

The first question to be determined was whether, in the earliest lesions, there could be shown a complete blockage of the capillaries

[1] *Johns Hopkins Hosp. Rep.*, 1895, 5, p. 379.
[2] *Johns Hopkins Hosp. Bull.*, 1892, 3, p. 17.
[3] *Jour. Exper. Med.*, 1897, 2, p. 197.
[4] *Ibid.*, 1898, 3, p. 611.

in the necrotic areas. Rats were infected by subcutaneous inoculation, and after varying intervals, india-ink was injected into the portal vein. In this way there was readily produced a diffuse and uniform injection of all the liver capillaries. The necrotic areas, however, remained completely uninjected, as shown in Fig. 6,

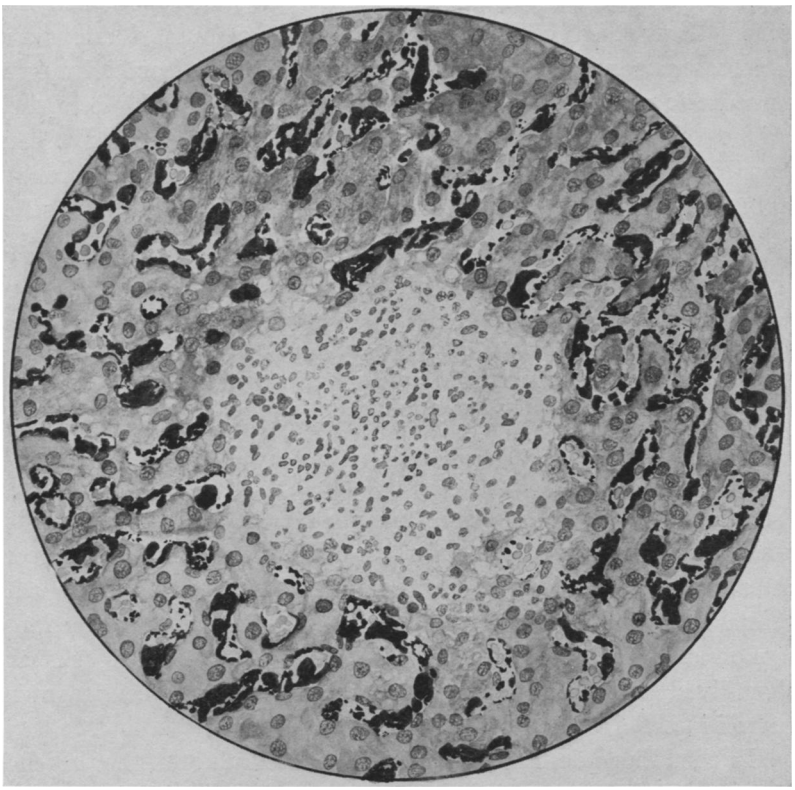

FIG. 6.—Liver of experimentally infected rat after injection of india-ink into portal vein. The necrotic area is impervious to the injection fluid.

and stood out conspicuously from the surrounding injected liver tissue. The capillaries in even the smallest and presumably the earliest necroses could be shown in serial sections to be absolutely impermeable to the injection fluid.

This fact, it seems to us, argues against a primary toxic injury to the epithelial cells, followed by a secondary thrombotic occlusion;

for, in such an event, one would expect to find lesions in which the capillaries were at least partially permeable to the injection fluid.

Because of the profound destruction of cells in the spleen, it seemed probable that the cell fragments found in the liver capillaries might be largely derived from this source. The fact that the intestinal lesions were relatively slight also seemed to bear out this idea. The same view had been previously expressed by Boxmeyer, who, in discussing the source of the large mononuclears found in the liver capillaries, stated that "in the mouse . . . . it is safe to conclude that by far the greater number arise in the spleen and are carried to the capillaries of the liver."

To test this point, we removed the spleens from two rats, infected them, together with controls, and killed them after three days. The liver necroses were of the same character in both sets of animals, and were associated with the formation of fibrin thrombi about cell-fragments. If anything, the necrotic areas were more numerous in the splenectomized rats than in the controls. From these experiments, it would seem that the presence of cells derived from the spleen is not essential for the development of the liver lesions.[1]

That necrotic cells, cell-fragments, and phagocytes are discharged from the spleen and are held back by the capillaries of the liver and spleen could be shown not only by finding such cells in masses in the afferent veins of the liver and in the pulmonary arteries, but by comparing smears made directly from the splenic vein with smears from the left ventricle.

The following count will serve as an example:

*Rat M-5-2.*—Injected subcutaneously. Killed after 57 hours. Typical gross and microscopic lesions.

| Smear from splenic vein: | Per cent |
|---|---|
| Polymorphonuclear neutrophiles | 26 |
| (Of these, 10 showed degeneration, i.e., vacuolization of cytoplasm, loss of granules, indistinct nuclear outline, pale staining of nucleus; some of the leukocytes contained bacilli.) | |
| Small lymphocytes | 11 |
| (Of these, 2 showed distinct pyknosis, with irregularity of nucleus.) | |
| Large lymphocytes | 14 |
| (Of these, 3 showed indistinct nuclear outlines, pale staining, etc.) | |

[1] Attempts to ligature the portal vein and thus to prevent the embolism of cells from the intestinal tract as well as the spleen, were unsuccessful, the rats surviving the operation only three to four hours.

| | Per cent |
|---|---|
| Large mononuclear cells | 18 |
| (Characterized by round or reniform nucleus with distinct nucleolus, abundant faintly basophilic cytoplasm often studded with purplish granules. Many of these cells inclose one or several bacilli. One cell found undergoing mitosis.) | |
| Degenerated cells, unclassified | 31 |
| (Degenerated cells of various types, some mononuclear, some apparently derived from swollen polynuclears. They often enclose more or less disintegrated bipolar bacilli and occasionally nuclear fragments.) | |
| Smear from heart blood (left ventricle): | |
| Polymorphonuclear neutrophiles | 34 |
| (Of these, 5 showed degeneration. Several inclosed one or several bacilli, one a red blood cell, one a nuclear fragment of normoblast or lymphocyte.) | |
| Small lymphocytes | 9 |
| Large lymphocytes | 31 |
| Large mononuclears (not degenerated) | 18 |
| Degenerated cells, unclassified | 8 |

In the smear from the splenic vein, no less than 46 per cent of all the leukocytes showed distinct evidence of degeneration, as against 13 per cent in the blood from the left ventricle. A similar difference was found in other infected rats. Altho the classification of many of the cells found in the smears may be open to question, the contrast is sufficiently striking to indicate that degenerated cells, and particularly phagocytes with bacillary or nuclear inclusions, pass out from the spleen and are held back by the capillaries of the liver and lungs from the general circulation—at least in part.

Since some of the phagocytes contain bacilli, it is difficult to say with certainty that the bacilli or their disintegration products are not concerned in the production of the liver lesions. Even if, as we believe, the lesion begins as a cell-fragment thrombus in the capillaries, it is readily conceivable that the death of the adjacent liver cells is due, not so much to a mechanical obstruction of the circulation, as to the toxic influence of bacilli included in the thrombosed area. Too much emphasis should not be laid upon the failure to find bacilli in the lesions, since the degeneration forms, particularly the intracellular fragments, are extremely difficult to identify with certainty in sections. If, however, identical lesions could be produced by the injection of sterile cellular suspensions into the portal circulation, it would exclude the bacterial factor as essential to the production of the liver lesions. To test this possibility, the spleen was removed aseptically from healthy rats, and a rather dense suspension made by teasing the tissue in sterile

Ringer's solution. This was freed from coarser particles, and, after varying periods, injected through a fine glass pipette, into the mesenteric veins of large rats. The injected animals were killed after intervals varying from three and one-half to 48 hours. The results of these experiments were inconclusive. Distinct lesions were found only in the liver of the rat killed after three and one-half hours. In this animal, they simulated so closely the lesions found in the bacillary infection that the suspicion arose that the rat had been spontaneously infected with the disease at the time of the experiment. Cultures, however, yielded only a few colonies of a white staphylococcus. The other four experiments were wholly negative.

This inconclusive result leads us to express with caution a decided view as to the pathogenesis of the lesions. Certain definite inferences may, however, be drawn from a study of the histological features of the disease. It is plain that the bacterial toxin, whatever its nature, brings about an intense destruction of leukocytes and lymphoid cells in all hematopoietic tissues—spleen, lymph-nodes and bone-marrow. Associated with this injury, there is an active proliferation of large mononuclear cells, many of which become phagocytic and in turn degenerate. In many of the organs, and most strikingly in spleen, liver, and lungs, fibrin thrombi are formed about masses of degenerating cells and cell fragments. In the liver where the structural relations are less complicated than in the spleen and bone-marrow, one may state with assurance that the formation of these cell-fragment fibrin thrombi antedates the destruction of the liver cells, and is the first step in the formation of the necrosis. This point, we believe, is clearly brought out by our ink-injection experiments, as well as by a careful study of the early experimental lesions, in which the thrombi may be found unassociated with necrosis of the liver cells.

Whether the liver cells become affected by the mechanical interference with the blood supply, or whether they degenerate because of the toxic effect of bacilli entrapped in the thrombus, or whether possibly they are poisoned by autolytic products from the impacted cells, it is not at present possible to decide.

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